

Supplemental Figure 1



Supplemental Figure 2





Supplemental Figure 4



Females





















CD3ε





RANTES





MCP-1





2 Supplemental Table 1: Tissue masses of 24 wk old mice.

| Genotype | Perirenal WAT (mg) | | Spleen (mg) | | Kidneys (mg) | |
|-------------------------------|--------------------|----------|-------------|---------|--------------|----------|
| | CD | WD | CD | WD | CD | WD |
| | | | | | | |
| Males | | | | | | |
| MIP-1α ^{+/+} | 68 ± 9 | 930 ± 76 | 72 ± 11 | 103 ± 6 | 341 ± 19 | 379 ± 13 |
| MIΡ-1α ^{+/-} | 92 ± 12 | 866 ± 39 | 63 ± 20 | 103 ± 4 | 324 ± 12 | 376 ± 9 |
| MIP-1α ^{-/-} | 72 ± 16 | 955 ± 41 | 71 ± 4 | 111 ± 6 | 339 ± 13 | 369 ± 9 |
| Females | | | | | | |
| MIΡ-1α ^{+/+} | 99 ± 19 | 622 ± 74 | 63 ± 2 | 117 ± 7 | 247 ± 11 | 285 ± 8 |
| MIΡ-1α ^{+/-} | 66 ± 5* | 587 ± 80 | 73 ± 5 | 111 ± 5 | 260 ± 8 | 273 ± 7 |
| <u></u> ΜΙΡ-1α ^{-/-} | 96 ± 13 | 566 ± 54 | 68 ± 5 | 118 ± 5 | 246 ± 9 | 262 ± 10 |

Data shown is mean ± SEM. *P<0.05 for one-way ANOVA, but Tukey's Multiple Comparison Test revealed no significant differences between MIP-1 $\alpha^{+/+}$, MIP-1 $\alpha^{+/-}$, and MIP-1 $\alpha^{-/-}$ female mice. Abbreviations: CD, chow diet; WD, Western Diet.

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1 Supplemental Figure 1: Energy expenditure relative to body mass in male MIP-1 $\alpha^{+/+}$, MIP-2 $1\alpha^{+/-}$, and MIP-1 $\alpha^{-/-}$ mice. Energy expenditure is plotted to include all time points at which data 3 was collected during a 24 h period (A). Data is plotted as mean ± SEM for each group during 4 the 12 h of light (left panel) or 12 h of dark (right panel) of the 24 h light/dark cycle (B). Mice 5 were weighed at the start of the experiment, and their energy expenditure was analyzed relative 6 to their body mass. Abbreviations: EE, energy expenditure. n=7-9 mice/group.

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8 Supplemental Figure 2: Daily food consumption of male mice after 8 wks on Western
9 Diet. Food consumption was measured while mice were individually housed during the energy
10 expenditure experiment. Change in food mass per hour was calculated and converted into
11 grams per day. n=7-9 male mice/group.

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Supplemental Figure 3: WAT gene expression in Western Diet-fed mice. Mice were fed
Western Diet for 16 wks. RNA was isolated from perigonadal WAT and used to synthesize
cDNA, which was used for real time PCR. Data shows the relative gene expression of
adiponectin (A-B), TNFα (C-D), arginase-1 (E-F), and IL-10 (G-H).

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Supplemental Figure 4: Toluidine blue O stained WAT in mice after 16 wks of Western Diet feeding. Perigonadal WAT was harvested from mice, weighed, and a portion was fixed overnight in 10% formalin, transferred to 70% ethanol, and paraffin embedded. Tissue was cut into 7µm sections and stained with toluidine blue O (Panels A-F) or immunostained with primary antibody to F4/80 (Panels G-L). Toluidine Blue O images were taken at 10X magnification and F4/80 stained images were taken at 20X magnification.

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Supplemental Figure 5: WAT gene expression in six week WD-fed mice. Mice were fed Western Diet for 6 wks. RNA was isolated from perigonadal WAT and used to synthesize cDNA, which was used for real time PCR. Data shows the relative gene expression of F4/80, CD68, CD3 ϵ , MIP-1 α , MIP-1 β , RANTES, and MCP-1. Data are from 8-9 females per group and 4 males per group and are expressed as mean ± SEM.

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Supplemental Figure 6: Flow cytometric analysis of stromal vascular fractions from WAT of 6 week WD-fed male mice. The stromal vascular fraction was isolated from WAT of mice fed the WD for 6 weeks and flow cytometric analysis performed as described in the Methods section. Macrophages and T lymphocytes were identified as F4/80 and CD3 positive cells quantified as a percent of the live cell population. Sub-populations of T lymphocytes were measured by quantifying the percent of CD3+ cells that were either CD4+ (helper T-lymphocytes) or CD8+ (cytotoxic T-lymphocytes).

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40 Supplemental Figure 7: Correlation between MCP-1 expression and macrophage 41 markers. Relative gene expression of F4/80 and CD68 are plotted versus MCP-1 relative gene 42 expression for individual mice after 16 wks on Western Diet. Data from male MIP-1 $\alpha^{-/-}$, MIP-43 $1\alpha^{+/-}$, and MIP-1 $\alpha^{+/+}$ mice have been analyzed together. Linear regression line is shown. *P* 44 values indicate the significance of each slope's deviation from zero.

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